

U.S. Patent Application No. 10/602,035
Amendment dated July 17, 2007
Reply to Office Action of April 18, 2007

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REMARKS/ARGUMENTS

Reconsideration and continued examination of the above-identified application are respectfully requested.

By way of this amendment, claims 1-32 are pending. Claims 33-36 have been canceled. Accordingly, no questions of new matter should arise and entry of this amendment is respectfully requested.

Election/Restriction Requirements

The Examiner has requested that the claims be amended to the elected/rejoined group, including the elected compound.

In response, the applicant will make such an amendment upon an indication of allowable subject matter.

Rejection of claims 33 and 34 under 35 U.S.C. §102(b) – Powers et al.

At page 2 of the Office Action, the Examiner maintains the rejection of claims 33 and 34 under 35 U.S.C. §102(b) as being anticipated by Powers et al. (U.S. Patent No. 5,543,396). The Examiner asserts that Powers et al. teaches a pharmaceutical composition in any form comprising the elected compound, and the Examiner makes reference to the "tissue remodeling" at col. 1, lines 41-43 of Powers et al. and appears to equate this "tissue remodeling" to include tissue adhesion formation. The rejection in its entirety is respectfully traversed.

This rejection is moot in view of the cancellation of claims 33-34.

Accordingly, this rejection should be withdrawn.

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Rejection of claims 1-34 under 35 U.S.C. §103(a) over Powers et al. in view of Scharpe et al.

At page 3 of the Office Action, the Examiner has maintained the rejection of claims 1-34 and new claims 35-36 under 35 U.S.C. §103(a) as being unpatentable over Powers et al. in view of Scharpe et al. (U.S. Patent Application Publication No. 2002/0061839 A1). The Examiner relies on Powers et al. as described above in the §102 rejection. The Examiner does acknowledge in this rejection that Powers et al. does not expressly teach the use of the elected peptide to reduce adhesion formation or the various forms of administration set forth in claims 25-30. However, the Examiner cites Porter et al. (U.S. Patent No. 5,591,199) to suggest that it is well-known in the art to use protease inhibitors to reduce adhesion formation. The Examiner relies on Scharpe et al. to assert that Scharpe et al. uses serine protease inhibitors in virtually any pharmaceutical mixture/formulation. This rejection in its entirety is respectfully traversed.

With respect to this §103 rejection, the applicant respectfully points out that Powers et al. does not teach or suggest a method of using a protease inhibitor to reduce adhesion formation, as presently claimed. Adhesion formation, many times occurs through surgical procedures, wherein the adhesion can result from a wound healing response. The present inventor has found that adhesion formation between tissue surfaces in a vertebrate subject can be reduced by administering to the subject an effective amount of a protease inhibitor to a site on a tissue surface. Applicant also points out that similar findings have been reported in the article ("Inhibition of Transforming Growth Factor- β Activation is a Novel Effect of Chymase Inactivation," 2005) submitted herewith, which states that "... TGF- β protein was significantly increased after the injection of chymase, but this increase in TGF- β was inhibited by a peptidic chymase inhibitor, Suc-Val-Pro-Phe^P (OPh)₂... TGF- β has been implicated in the pathogenesis of several diseases, including adhesion, ..." See page 19, second column.

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The primary purpose of Powers et al., on the other hand, is to use certain derivatives as anti-coagulants, anti-inflammatory agents, and anti-tumor agents. Powers et al. makes reference to tissue remodeling, but tissue remodeling is not equivalent or a genus of treating adhesion formation. The applicant also points out that Powers et al. does not teach or suggest that tissue remodeling encompasses tissue adhesion formation. In addition, one skilled in the art would readily recognize the difference between tissue remodeling and tissue adhesion formation. Tissue remodeling generally involves the remodeling or rebuilding of damaged tissue, which, many times, is carried out through changing the formation of the tissue itself. Adhesion formation is quite different from tissue remodeling. As described at the bottom of page 1 and top of page 2 of the present application, adhesion formation and adhesion-free re-epithelialization are alternative pathways, both of which begin with coagulation and which can result in the build-up of fibrin gel matrix, and if this fibrin deposition is in excess or not removed, the gel matrix serves as a progenitor to adhesions by forming a band or bridge when two tissue surfaces coated with fibrin matrix are apposed. Accordingly, the applicant respectfully submits that use of protease inhibitors for reducing adhesion formation between tissue surfaces differs from use of protease inhibitors for anti-coagulant, anti-inflammatory, and tissue remodeling purposes.

The applicant also points out that the Examiner's suggestion that Porter et al. teaches that protease inhibitors and anti-inflammatory agents are used for reducing tissue adhesion is incorrect. Porter et al. teaches that thiol protease inhibitors and anti-inflammatory agents can be used to treat vascular disease only (col. 5-6).

The applicant also notes that Porter et al. states that anticoagulants can prevent platelet adhesion. However, as is generally known, platelet adhesion differs from tissue adhesion. Thus, Porter et al. also cannot be relied upon to establish that anticoagulants are useful for preventing

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tissue adhesion.

In addition, the applicant respectfully points out that Scharpe et al. does not overcome the deficiencies of Powers et al. Scharpe et al. does not teach or suggest treating adhesion formation. Furthermore, neither Powers et al. nor Scharpe et al. teach or suggest applying a protease inhibitor to a site on a tissue surface to reduce adhesion formation, as recited in claims 1 and 16. The applicant also points out that neither Powers et al. nor Scharpe et al. teach or suggest administering a protease inhibitor in conjunction with a delivery vehicle that maintains an effective local concentration of the protease inhibitor at a site on the tissue surface, as recited in claims 25-30.

Further, it is unclear whether one skilled in the art would even look to Scharpe et al. to modify the compound of Powers et al. when Powers et al. makes no teaching or suggestion to make such a modification and, further, the particular administration routes set forth in Powers et al. are very clear with respect to use of the derivatives of Powers et al. as anti-coagulants, anti-inflammatory agents, and anti-tumor agents.

Accordingly, this rejection should be withdrawn.

Provisional Rejection -- Obviousness-Type Double Patenting

At page 6 of the Office Action, the Examiner provisionally rejects claims 1-34 on the ground of obviousness-type double patenting as being unpatentable over claims 1-20 of co-pending U.S. Patent Application No. 10/544,254. This provisional rejection is respectfully traversed.

Since this is a provisional rejection, once the remaining rejections described above have been overcome, this provisional rejection should be withdrawn and, if necessary, applied in co-pending U.S. Patent Application No. 10/544,254.

Accordingly, this provisional rejection should be withdrawn once it is the only remaining

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rejection in the present application.

Rejection of claims 1-34 under 35 U.S.C. §112, first paragraph

At page 7 of the Office Action, the Examiner rejects claims 33-36 under 35 U.S.C. §112, first paragraph, for enablement reasons. The Examiner believes that reference to "preventing" adhesion formation is not enabled by the present application. This rejection is respectfully traversed.

Applicant believes that the application is enabling for the scope provided in the pending claims. To assist the Examiner, claims 33-36 have been canceled.

Accordingly, this rejection should be withdrawn.

Objections to claim numbering

With respect to claims 25-30, the applicant will be willing to re-arrange the numbering of the claims upon the allowability of the subject matter of the present application.

Request For Telephone Interview

The undersigned requests a telephone interview to discuss the above comments upon the Examiner's review of this response. The undersigned can be reached at the number below.

CONCLUSION

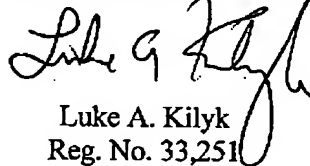
In view of the foregoing remarks, the applicant respectfully requests the reconsideration of this application and the timely allowance of the pending claims.

If there are any fees due in connection with the filing of this response, please charge the fees

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to our Deposit Account No. 50-0925. If a fee is required for an extension of time under 37 C.F.R. §
1.136 not accounted for above, such extension is requested and should also be charged to said
Deposit Account.

Respectfully submitted,


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Enclosure: Takai et al., "Inhibition of Transforming Growth Factor- β
Activation is a Novel Effect of Chymase Inactivation,"
LETTERS IN DRUG DESIGN & DISCOVERY, 2, p. 19-22, 2005.

Inhibition of Transforming Growth Factor- β Activation is a Novel Effect of Chymase Inactivation

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Abstract: Chymase activates latent transforming-growth factor- β to transforming-growth factor- β *in vitro*. Recent papers demonstrate that transforming-growth factor- β levels and tissue fibrosis were significantly reduced by chymase inhibitors in the experimental models. Thus, transforming-growth factor- β -related diseases such as fibrosis may become a novel target of chymase inhibitors.

Keywords: Adhesion, chymase, inhibitor, fibrosis, transforming growth factor- β .

SIGNIFICANCE OF CHYMASE-DEPENDENT ANGIOTENSIN II FORMATION

Chymase is a chymotrypsin-like serine protease contained in the secretory granules of mast cells. Chymase has been known to activate angiotensin I to form angiotensin II [1 - 5]. Angiotensin II plays an important role in vascular proliferation in addition to blood pressure. An ARB was successful in preventing restenosis after percutaneous transluminal coronary angioplasty in a clinical study, but an ACE inhibitor was not [6-9]. In rat vascular tissues, ACE is the only angiotensin II-forming enzyme, while the vascular tissues of humans contain chymase in addition to ACE as angiotensin II-forming enzymes [10]. In general, chymase is a chymotrypsin-like enzyme and hydrolyzes the C-terminal side of proteins after aromatic amino acids such as Phe, Tyr, and Trp. Angiotensin I contains two aromatic amino acids, Tyr and Phe. However, human, monkey, dog and hamster chymases cleave the Phe⁸-His⁹ bond of angiotensin I to yield angiotensin II [11]. Therefore, an ACE inhibitor could not suppress chymase-dependent angiotensin II formation, resulting in vascular proliferation in human vessels. In a dog balloon-injury model, both ACE and chymase activities were increased, with the increase in chymase activity being greater than that of ACE activity after the operation [12 - 14]. In this model, an ARB or a chymase inhibitor significantly suppressed the formation of intimal hyperplasia in the injured arteries, while an ACE inhibitor did not [13, 14]. These reports strongly indicate that chymase-dependent angiotensin II formation plays a crucial role in the development of vascular proliferation.

ACTIVATION OF TRANSFORMING-GROWTH FACTOR- β BY CHYMASE *IN VITRO*

Chymase may contribute to the activation of transforming-growth factor (TGF)- β [15, 16]. TGF- β is released from a latent TGF- β -binding protein in fibroblasts [17]. The latent TGF- β -binding protein is cleaved as latent

TGF- β , and the latent form of TGF- β is activated to TGF- β by extremes of pH and by plasmin [18 - 20]. Taipale *et al.* [15] suggested that chymase could contribute to the release of latent TGF- β from latent TGF- β -binding proteins of the extracellular matrix of human epithelial and endothelial cells. In human dermal fibroblasts, chymase was found to significantly increase cell proliferation in fibroblasts [16]. This increased cell proliferation was completely suppressed by a chymase inhibitor, but not by an ARB [16]. In media supernatants of cultured fibroblasts, the concentration of TGF- β protein was significantly increased after the injection of chymase, but this increase in TGF- β was inhibited by a peptidic chymase inhibitor, Suc-Val-Pro-Phe(OPh)₂. Anti-TGF- β neutralizing antibody completely suppressed cell proliferation induced by human chymase, indicating that chymase induced the cell proliferation through TGF- β activation. On the other hand, TGF- β could induce the cell proliferation in human fibroblasts, but latent TGF- β could not. As shown in (Fig. 1), chymase cleaves not only a latent TGF- β -binding protein in fibroblasts to a latent TGF- β , but also a latent TGF- β to active TGF- β , resulting in inducing cell proliferation of fibroblasts. TGF- β has been implicated in the pathogenesis of several diseases including adhesion, cardiomyopathy and pulmonary fibrosis, and the chymase-dependent TGF- β activation may contribute to the development of the pathogenesis *in vivo*.

ROLE OF CHYMASE ON ADHESION AFTER SURGERY

Postoperative adhesions are well-known complications of surgery. Mast cells are known to be inflammatory cells, and previous reports suggest that mast cells may be involved in peritoneal inflammation and adhesion [21 - 24]. The number of mast cells is increased around wounds in the late stages of the healing process [21, 22]. In contrast, mast-cell stabilizers, which inhibit the activation and accumulation of mast cells, are effective in attenuating adhesion formation in rat models [21, 23]. In mast-cell-deficient mice, adhesion formation was significantly less severe than that in normal control mice [25]. These reports suggest that mast cells are involved in adhesion formation. However, mast cells release a large number of inflammatory mediators such as histamine, serotonin, chemotactic factors, cytokines and serine proteases

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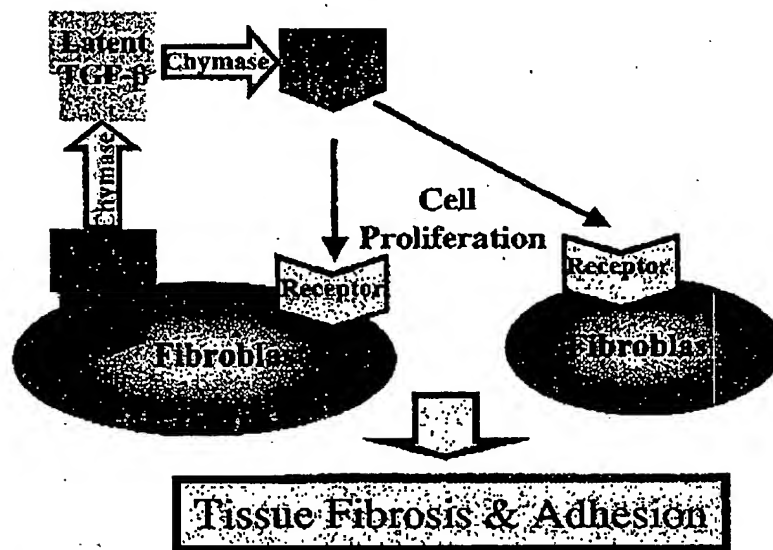


Fig. (1). Role of chymase on developments of tissue fibrosis and adhesion *via* TGF- β activation from fibroblasts.

during the repair phase of adhesion formation [25 - 28], and it has been unclear which factors play important roles in the development of adhesion formation.

Cheginini found evidence for a key role for TGF- β in adhesion formation [29]. In a rat model, although intact peritoneal/fascial tissue contains a very low level of TGF- β , the level of TGF- β was significantly increased within the fibrosis adhesion after peritoneal wall injury [30, 31]. In a mouse adhesion model, the level of TGF- β in peritoneal fluid was significantly higher during the first week postsurgery than in uninjured controls [31]. In fact, the intraperitoneal injection of TGF- β accelerates adhesion formation [30]. In contrast, the intraperitoneal injection of a neutralizing antibody to TGF- β decreased adhesion formation in a rat adhesion model [32, 33]. These reports suggest that TGF- β may play an important role in the development of adhesion formation.

In a hamster adhesion model, hamsters were received a lesion produced by uterus scraping, and a peptidic chymase inhibitor, Suc-Val-Pro-Phe²(OPh)₂, or placebo was injected into the abdomen. In the placebo-treated hamsters, chymase activities in the injured uteri were significantly increased from 3 days after the operation and the adhesion formations were observed at 2 weeks, while not only the chymase activities and adhesion formation was significantly reduced in the chymase inhibitor-treated hamsters [34]. In this adhesion model, TGF- β concentrations in peritoneal fluid were significantly increased after scraping the uterus, while the increased TGF- β concentrations were reduced by treatment with Suc-Val-Pro-Phe²(OPh)₂ [35]. Recently, we evaluated the effect of Suc-Val-Pro-Phe²(OPh)₂ on scarring in a canine conjunctival flap model [36]. In the canine surgical model, the chymase activity, adhesion degree and mast cell number were significantly increased in the operated eyes, while they were significantly decreased by treatment with the

chymase inhibitor. On the other hand, oral administrations of non-peptidic inhibitors, BCEAB and NK3201, were also useful for prevention of adhesion formation in experimental adhesion models [37, 38]. Therefore, chymase inhibition might be related to the reduction of TGF- β activation *in vivo*, and its mechanism may play an important role in preventing adhesion formation after surgery.

ROLE OF CHYMASE ON CARDIOMYOPATHY

In cardiac tissue of cardiomyopathic patients and hamsters, TGF- β , which is a well-known growth factor that stimulates fibrosis *via* the accumulation of extracellular matrix, is increased [39, 40]. Mast cells are found in increased numbers in the myocardial fibrotic area in cardiomyopathy, and this increase in the number of cardiac mast cells may contribute to the development of fibroblast proliferation in cardiac tissues of cardiomyopathy [41, 42].

In a hamster model of cardiomyopathy, mast cells number and chymase activities in cardiac tissues were significantly increased along with cardiac fibrosis. The expression of collagen I and collagen III genes were also significantly increased in the cardiac tissues [43]. Increased collagen synthesis may play an important role in impairing cardiac function in the development of cardiomyopathy. TGF- β is known to induce the expression of collagen I and collagen III genes [44]. However, a non-peptidic chymase inhibitor, BCEAB, significantly suppressed the chymase activity, mRNA levels and the fibrotic area in heart, resulted in prevention of cardiac dysfunction [45]. However, the chymase inhibitor could not reduce the cardiac hypertrophy. Kuwahara *et al.* [46] reported that the administration of anti-TGF- β neutralizing antibody prevented both the expression of collagen genes and cardiac fibrosis, but not cardiac hypertrophy. Gene expression of TGF- β is known to be induced by angiotensin II [47, 48]. Chymase can produce

Inhibition of Transforming Growth Factor- β

angiotensin II from angiotensin I, and this angiotensin II produced by chymase may be involved in the pathogenesis of cardiac fibrosis in cardiomyopathic hamsters. In fact, an ARB could also prevent fibrotic formation in cardiomyopathic hamsters [47, 48]. An ARB reduced the cardiac hypertrophy in the hamster model of cardiomyopathy in addition to reducing the expression of collagen genes and cardiac fibrosis, while a chymase inhibitor did not effect the cardiac hypertrophy. The difference between ARB and chymase inhibitor relative to the effect on the cardiac hypertrophy suggests different mechanisms in their improvement of cardiac function. Therefore, increases in cardiac chymase activity in cardiomyopathy may induce TGF- β activation, and this may play an important role in inducing cardiac fibrosis and dysfunction via induction of the expression of collagen I and collagen III genes.

ROLE OF CHYMASE ON PULMONARY FIBROSIS

In idiopathic pulmonary fibrosis in patients and animals, TGF- β is increased [49, 50]. In animal models of bleomycin-induced pulmonary fibrosis, TGF- β may play an important role in the development of pulmonary fibrosis. For example, administration of anti-TGF- β antibodies or an antagonist of TGF- β signaling could reduce bleomycin-induced pulmonary fibrosis via reduction of collagen mRNA levels [51, 52]. Chymase is contained in granules of mast cells, and mast cells are found in increased numbers in the pulmonary tissues of mice with bleomycin-induced pulmonary fibrosis [53]. In a mice experimental model, this fibrosis was suppressed by the treatment with a mast cell stabilizer [54]. Therefore, mast cells may also contribute to the development of pulmonary fibrosis [53, 55]. In a hamster experimental model, both chymase activity and fibrotic area in pulmonary tissues were significantly increased after the treatment with bleomycin. Treatment with NK3201 significantly decreased not only chymase activity but also the fibrotic area [56, 57]. The increased mRNA level of collagen III after treatment with bleomycin was also significantly reduced by treatment with NK3201. These findings clearly suggest that an increase in chymase activity may be involved in the development of bleomycin-induced pulmonary fibrosis in hamsters.

CONCLUSIONS

Chymase functions as a TGF- β -activating enzyme in fibroblasts, and inhibition of this function may represent a novel target for prevention of adhesion, cardiomyopathy and pulmonary fibrosis.

REFERENCES

- [1] Urata, H.; Kinoshita, A.; Misono, K.S.; Bumpus, F.M.; Husain, A. *J. Biol. Chem.*, 1990, 265, 22348-22357.
- [2] Takai, S.; Shiota, N.; Sakaguchi, M.; Muraguchi, H.; Matsumura, E.; Miyazaki, M. *Clin. Chim. Acta*, 1997, 265, 13-20.
- [3] Takai, S.; Shiota, N.; Kobayashi, S.; Matsumura, E.; Miyazaki, M. *FEBS Lett.*, 1997, 412, 86-90.
- [4] Caughey, G.H.; Raymond, W.W.; Wolters, P.J. *Biochim. Biophys. Acta*, 2000, 1480, 245-257.
- [5] Takai, S.; Shiota, N.; Yamamoto, D.; Okunishi, H.; Miyazaki, M. *Life Sci.*, 1996, 58, 591-597.
- [6] Powell, J.S.; Clozel, J.P.; Muler, R.K.M.; Kuhn, H.; Hefti, F.; Hoesang, M.; Baumgartner, H.R. *Science*, 1991, 245, 186-188.
- [7] Kauffman, R.P.; Bean, J.S.; Zimmerman, K.M.; Brown, R.F.; Steinberg, M.I. *Life Sci.*, 1991, 49, PL223-PL228.
- [8] MERCATOR Study Group. *Circulation*, 1992, 86, 100-110.
- [9] Peters, S.; Gotting, B.; Trummel, M.; Rust, H.; Brattstrom, A. *J. Invasive. Cardiol.*, 2001, 13, 93-97.
- [10] Takai, S.; Sakaguchi, M.; Jin, D.; Yamada, M.; Kirimura, K.; Miyazaki, M. *Clin. Chim. Acta*, 2001, 305, 191-195.
- [11] Takai, S.; Miyazaki, M. *Jpn. J. Pharmacol.*, 2002, 90, 223-227.
- [12] Miyazaki, M.; Shiota, N.; Sakonjo, H.; Takai, S. *Jpn. J. Pharmacol.*, 1999, 79, 455-460.
- [13] Miyazaki, M.; Wada, T.; Shiota, N.; Takai, S. *J. Hum. Hypertens.*, 1999, 13 (Suppl. 1), S21-S25.
- [14] Takai, S.; Sakonjo, H.; Fukuda, K.; Jin, D.; Sakaguchi, M.; Kamoshita, K.; Ishida, K.; Sukenaga, Y.; Miyazaki, M. *J. Pharmacol. Exp. Ther.*, 2003, 304, 841-844.
- [15] Taipale, J.; Lohi, J.; Saurinen, J.; Kovacs, P.T.; Keski-Oja, J. *J. Biol. Chem.*, 1995, 270, 4689-4896.
- [16] Takai, S.; Jin, D.; Sakaguchi, M.; Katayama, S.; Muramatsu, M.; Sakaguchi, M.; Matsumura, E.; Kim, S.; Miyazaki, M. *J. Pharmacol. Exp. Ther.*, 2003, 305, 17-23.
- [17] Kanazaki, T.; Olofsson, A.; Morén, A.; Wernstedt, C.; Hellman, U.; Miyazono, K.; Claesson-Welsh, L.; Heldin, C.H. *Cell*, 1990, 61, 1051-1061.
- [18] Lawrence, D.A.; Pircher, R.; Jullien, P. *Biochem. Biophys. Res. Commun.*, 1985, 133, 1026-1034.
- [19] Lyons, R.M.; Gentry, L.E.; Purchio, A.P.; Moses, H.L. *J. Cell. Biol.*, 1990, 110, 1361-1367.
- [20] Miyazono, K.; Heldin, C.H. *Nature*, 1989, 338, 158-160.
- [21] Persinger, M.A.; Lepage, P.; Simard, J.P.; Parker, G.H. *Br. J. Dermatol.*, 1983, 108, 179-187.
- [22] Lieberman, S.M.; Langer, J.C.; Marshall, J.S.; Collins, S.M. *Am. J. Surg.*, 1993, 165, 127-130.
- [23] Langer, J.C.; Lieberman, S.M.; Monk, P.K.; Pelletier, G.J. Mast cell mediators and peritoneal adhesion formation in the rat. *J. Surg. Res.*, 1995, 59, 344-348.
- [24] Adachi, S.; Maruyama, T.; Kondo, T.; Todoroki, T.; Fukui, K. *Surg. Today*, 1999, 29, 51-54.
- [25] Yao, Y.L.; Ishihara, T.; Takai, S.; Miyazaki, M.; Mita, S. *J. Surg. Res.*, 2000, 92, 40-44.
- [26] Metcalfe, D.D. *Allergy*, 1984, 33, 563-575.
- [27] Ramos, B.F.; Zhang, Y.; Jakisch, B.A.; Qureshi, R. *J. Immunol.*, 1991, 147, 1636-1641.
- [28] Das, A.M.; Flower, R.J.; Perreth, M. *J. Immunol.*, 1997, 159, 1466-1473.
- [29] Chegini, N. *Eur. J. Surg. (Suppl.)*, 1997, 377, 17-23.
- [30] Williams, R.S.; Rossi, A.M.; Chegini, N.; Schultz, G. *J. Surg. Res.*, 1992, 52, 65-70.
- [31] Chegini, N.; Gold, L.L.; Williams, R.S.; Masterson, B. *J. Obstet. Gynecol.*, 1994, 83, 449-454.
- [32] Lucas, P.A.; Warejcka, D.J.; Young, H.E.; Lee, B.Y. *J. Surg. Res.*, 1996, 65, 135-138.
- [33] Crowe, M.J.; Doetschman, T.; Greenhalgh, D.G. *J. Invest. Dermatol.*, 2000, 115, 3-11.
- [34] Okamoto, Y.; Takai, S.; Yamada, M.; Miyazaki, M. *Fertil. Steril.*, 2002, 77, 1044-1048.
- [35] Soga, Y.; Takai, S.; Koyama, T.; Okamoto, Y.; Ikeda, T.; Nishimura, K.; Miyazaki, M.; Komeda, M. *J. Thorac. Cardiovasc. Surg.*, 2004, 127, 72-78.
- [36] Maruichi, M.; Takai, S.; Sugiyama, T.; Ueki, M.; Oku, H.; Sakaguchi, M.; Okamoto, Y.; Muramatsu, M.; Ikeda, T.; Miyazaki, M. *Exp. Eye Res.*, 2004, 79, 111-118.
- [37] Okamoto, Y.; Takai, S.; Miyazaki, M. *Jpn. J. Pharmacol.*, 2002, 90, 94-96.
- [38] Okamoto, Y.; Takai, S.; Miyazaki, M. *J. Surg. Res.*, 2002, 107, 219-22.
- [39] Eghball, M.; Tomck, R.; Woods, C.; Bhambhani, B. *Proc. Natl. Acad. Sci. U. S. A.*, 1991, 88, 793-799.
- [40] Li, R.K.; Li, G.; Mickie, D.A.; Weiss, R.D.; Merante, F.; Luss, H.; Rao, V.; Christakis, G.T.; Williams, W.G. *Circulation*, 1997, 96, 874-881.
- [41] Panizo, A.; Mindan, F.J.; Galindo, M.F.; Conarrubalcilla, E.; Hernandez, M.; Dicz, J. *J. Hypertens.*, 1995, 13, 1203-1208.
- [42] Patella, V.; Marino, I.; Arbustini, E.; Lamparter-Schummert, B.; Verga, L.; Adt, M.; Marone, G. *Circulation*, 1998, 97, 971-978.
- [43] Dixon, I.M.; Ju, H.; Reid, N.L.; Scammell-La Fleur, T.; Werner, J.P.; Jansin, G. *J. Mol. Cell. Cardiol.*, 1997, 29, 1837-1850.

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- [44] Lijnen, P.J.; Petrov, V.V.; Fagard, R.H. *J. Renin Angiotensin Aldosterone Syst.*, 2001, 2, 117-122.
- [45] Kuwahara, F.; Kai, H.; Tokuda, K.; Kai, M.; Takeshita, A.; Egashira, K.; Imaizumi, T. *Circulation*, 2002, 106, 130-135.
- [46] Takai, S.; Jin, D.; Sakaguchi, M.; Katayama, S.; Muramatsu, M.; Sakaguchi, M.; Matsumura, E.; Kim, S.; Miyazaki, M. *J. Pharmacol. Exp. Ther.*, 2003, 305, 17-23.
- [47] Nakamura, F.; Nagano, M.; Kobayashi, R.; Higaki, J.; Mikami, H.; Kawaguchi, N.; Onishi, S.; Ogihara, T. *Am. J. Physiol.*, 1994, 267, H2297-H2304.
- [48] Taniyama, Y.; Morishita, R.; Hirata, K.; Aoki, M.; Nakagami, H.; Yamasaki, K.; Matsumoto, K.; Nakamura, T.; Kaneda, Y.; Ogihara, T. *Circulation*, 2000, 102, 246-252.
- [49] Brockelmann, T.J.; Limper, A.H.; Colby, T.V.; McDonald, J.A. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. *Proc. Natl. Acad. Sci. U. S. A.*, 1991, 88, 6642-6646.
- [50] Zhang, H.Y.; Gharace-Kermani, M.; Zhang, K.; Karmiol, S.; Pfan, S.H. Lung fibroblast alpha-smooth muscle actin expression and contractile phenotype in bleomycin-induced pulmonary fibrosis. *Am. J. Pathol.*, 1996, 148, 527-537.
- [51] Giff, S.N.; Hyde, D.M.; Hollinger, M.A. *Thorax*, 1993, 48, 959-966.
- [52] Nakao, A.; Fujii, M.; Matsumura, R.; Kumano, K.; Saito, Y.; Miyazono, K.; Iwamoto, I. *J. Clin. Invest.*, 1999, 104, 5-11.
- [53] Goto, T.; Befus, D.; Low, R.; Bienenstock, J. Mast cell heterogeneity and hyperplasia in bleomycin-induced pulmonary fibrosis of rats. *Am. Rev. Respir. Dis.*, 1984, 130, 797-802.
- [54] Mori, H.; Kawada, K.; Zhang, P.; Ucsugi, Y.; Sakamoto, O.; Koda, A. Bleomycin-induced pulmonary fibrosis in genetically mast cell-deficient WBB6F1-W/Wv mice and mechanism of the suppressive effect of tranilast, an antiallergic drug inhibiting mediator release from mast cells, on fibrosis. *Int. Arch. Allergy Appl. Immunol.*, 1991, 95, 195-201.
- [55] Tomioka, M.; Goto, T.; Lee, T.D.; Bienenstock, J.; Befus, A.D. Isolation and characterization of lung mast cells from rats with bleomycin-induced pulmonary fibrosis. *Immunology*, 1989, 66, 439-444.
- [56] Sukenaga, Y.; Kamoshita, K.; Takai, S.; Miyazaki, M. *Jpn. J. Pharmacol.*, 2002, 90, 218-222.
- [57] Sakaguchi, M.; Takai, S.; Jin, D.; Okamoto, Y.; Muramatsu, M.; Kim, S.; Miyazaki, M. A specific chymase inhibitor, NK3201, suppresses bleomycin-induced pulmonary fibrosis in hamsters. *Eur. J. Pharmacol.*, 2004, 493, 173-176.